ORIGINAL ARTICLE

Polymorphism of the Vitamin D Receptor Gene, Bone Mineral Density, and Bone Turnover in Postmenopausal Women from Lower Silesia (Poland)

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SUMMARY

Objective: Genetic factors are involved in determining bone mineral density (BMD) and in the pathogenesis of osteoporosis. There are relationships between various genetic polymorphisms and BMD or bone turnover. The aim of our study was to assess polymorphisms in the vitamin D receptor (VDR) gene (*BsmI*) in relation to BMD and bone turnover in a group of Polish postmenopausal women from the Lower Silesia region.

Materials and Methods: BMD at the lumbar spine and proximal femur, bone turnover (osteocalcin and carboxyterminal cross-linked telopeptide of type I collagen, ICTP), and restriction fragment length polymorphism (RFLP) of the VDR gene using the BsmI restriction enzyme were examined in 116 postmenopausal women.

Results: Despite the fact that we failed to detect statistically significant differences between the VDR genotypes in BMD, a trend towards higher BMD in women carrying allele b compared with wild-type subjects in our study is similar to previous reports. We also observed a higher allele b frequency in the control group (normal bone mass) compared with osteopenic/osteoporotic women (59.1% vs 40.0%). The lower activity of the resorption marker ICTP seen in allele b carriers could be involved.

Conclusion: We report an association between VDR gene polymorphism and decreased BMD in Polish postmenopausal women from the Lower Silesia region, but this requires further robust studies for confirmation. [*Taiwanese J Obstet Gynecol* 2005;44(1):57–61]

Key Words: BMD, bone turnover, osteoporosis, *VDR* gene polymorphisms

Introduction

Osteoporosis has become one of the most important public health problems due to fractures and their medical and economic consequences for aging individuals and society. Since osteoporosis can be influenced by hormonal status, dietary habits, lifestyle, and hereditary factors, it seems relevant to study the impact of these factors on bone mineral density (BMD) and bone turnover [1–3].

Genetic factors are involved in determining BMD and in the pathogenesis of osteoporosis. These are important in the peak bone mass attained and can impact bone turnover. Twin and family studies suggest that up to 85% of the variance in BMD could be genetically determined [4–6]. Other important factors are physical activity and hormonal and nutritional influences [3–7].

Several common allelic variants have been identified in the vitamin D receptor (VDR) gene. *BsmI* restriction fragment length polymorphisms (RFLPs) may be found in the non-coding region of the VDR gene in the intron separating exons 8 and 9. VDR gene polymorphism has been associated with peak bone mass [8] and higher rates of bone turnover [9] in adults and intestinal calcium absorption and BMD in growing children [10]. However, other studies have shown no association between VDR, ER and/or COLIA 1 gene polymorphisms and BMD or bone turnover [9,11–13].

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The aim of our study was to assess polymorphisms of the *VDR* gene in relation to BMD and bone turnover in a group of Polish postmenopausal women.

Materials and Methods

Subjects

We recruited by advertisement a group of healthy postmenopausal women who had not used hormone replacement therapy at any time. Menopause was defined as lack of menstrual bleeding for longer than 2 years. The study protocol was approved by the local ethics committee. All subjects gave their informed consent.

Analysis of the VDR gene polymorphism

VDR gene polymorphism (*BsmI*) was explored using polymerase chain reaction (PCR)-RFLP. Genomic DNA was obtained from human leukocyte nuclei isolated from whole blood. PCR products were digested using the restriction enzyme BsmI and then separated by polyacrylamide gel electrophoresis. The allele without the restriction site was designated the B allele and the allele with this restriction enzyme site was designated the b allele.

Bone mineral density

Densitometry of the lumbar spine (L2–L4) and proximal femur including the femoral neck, trochanter major, and Ward's triangle was carried out using dual-energy X-ray absorptiometry (DXA) with the Lunar DPX-plus densitometer (Lunar Co, Madison, WI, USA). The coefficient of variation (CV%) for DXA measurements at the four sites were 0.89%, 1.25%, 2.51%, and 2.82%, respectively.

Bone turnover markers

Serum osteocalcin concentration was analyzed using an immunoradiometric method (ELSA-OSTEO, CIS Biointernational, ORIS Group, Gif-Sur-Yvette, France) and carboxyterminal cross-linked telopeptide of type I collagen (ICTP) concentrations were determined using radioimmunoassay (Orion Diagnostica, Espoo, Finland). Intra- and interassay CV% were 4.7% and 4.9% for ICTP and 3.8% and 4.5% for osteocalcin, respectively.

Statistical analysis

Characteristics of the group variables are expressed as the mean and standard deviation. Allelic frequencies were estimated by gene counting, and genotype distribution of the polymorphisms was tested against the Hardy-Weinberg equilibrium by Chi-squared analysis. Allelic frequencies were compared between populations using Chi-squared analysis. Where appropriate, b allele homozygotes were combined with heterozygotes and the difference in clinical characteristics with and without the b allele was compared using the ANOVA test, adjusting for potential confounding factors (age, height, weight, years since menopause, tobacco smoking). All calculations were performed using statistical software (version 4.5 win, StatSoft Inc, Tulsa, OK, USA). The differences were statistically significant at a p value of 0.05 or less. In one case, borderline statistical significance of between 0.05 and 0.1 was reported.

Results

The study was carried out in 116 healthy postmenopausal women aged 52.4 ± 5.1 years who were 4.7 ± 3.6 years postmenopause. Most had a normal BMD at the examined sites (91 women), 20 women were osteopenic, and only five were osteoporotic according to the World Health Organization criteria. There were no differences in age, height, weight, and body mass index between these women (Table 1).

In order to compare clinical, biochemical, and genetic data between the groups with normal and decreased BMD, subjects with a normal BMD served as controls for osteopenic and osteoporotic subjects. Similar BMD values at various sites (expressed in T-score and Z-score) confirmed appropriate subject qualification for the groups with either normal or decreased BMD (Table 1).

PCR-RFLP revealed 19 wild type (homozygous BB) subjects (16.3%), 66 heterozygous allele b carriers (Bb genotype; 56.8%), and 31 homozygous allele b (bb genotype) subjects (26.7%). The allelic frequency of the b allele was calculated as 54.9%. Genotype distributions did not differ from those expected under Hardy-Weinberg equilibrium conditions (p = 0.14). In spite of the small number of subjects in the study, we attempted to compare allele b frequencies between subjects with a normal BMD or osteopenia/osteoporosis. There was a significantly lower frequency of *VDR* gene *BsmI* polymorphism in subjects with osteopenia and osteoporosis compared with control subjects (59.1% vs 40.0%; p = 0.005) (Table 1).

In a study of the influence of *BsmI* polymorphism on BMD and other anthropometric and clinical parameters, we found no statistically significant differences in age, BMD, and bone turnover parameters between subgroups with different VDR genotypes. Only bone resorption marker ICTP activity showed lower values (at the bor-

Table 1. Differences in clinical, biochemical, and genetic data between subjects with at least osteopenia (T-score < –1.0 standard deviations) and those with normal bone mineral density (BMD; control group)

	Control group	Osteopenia/osteoporosis	*p*
n	91	25	
Age (yr)	52.9 ± 6.7	51.5 ± 3.7	0.1
Height (cm)	163.1 ± 8.4	162.0 ± 11.7	0.6
BMI (kg/m^2)	29.3 ± 7.8	29.0 ± 8.9	0.4
Lumbar spine L2–L4			
BMD (g/cm^2)	1.307 ± 0.147	0.982 ± 0.117	0.000001
T-score	0.850 ± 1.259	–1.811 ± 0.971	0.000002
Z-score	0.843 ± 1.291	–1.070 ± 0.927	0.0003
Femoral neck			
BMD (g/cm^2)	0.995 ± 0.122	0.841 ± 0.089	0.001
T-score	0.128 ± 1.019	–1.155 ± 0.747	0.001
Z-score	0.265 ± 1.047	–0.403 ± 0.922	0.1
Ward's triangle			
BMD (g/cm^2)	0.876 ± 0.148	0.707 ± 0.106	0.004
T-score	0.258 ± 1.143	–1.555 ± 0.820	0.004
Z-score	–0.033 ± 1.172	–0.548 ± 0.853	0.1
Trochanter major			
BMD (g/cm^2)	0.849 ± 9.118	0.706 ± 0.072	0.002
T-score	0.544 ± 1.078	–0.760 ± 0.658	0.002
Z-score	0.466 ± 1.126	–0.391 ± 0.702	0.04
ICTP (ng/mL)	3.0 ± 1.2	2.1 ± 0.9	0.5
Osteocalcin (ng/mL)	18.6 ± 6.8	22.9 ± 3.8	0.1
Allelic frequency, *f(b)*	59.1%	40.0%	0.005

BMI = body mass index; ICTP = carboxyterminal cross-linked telopeptide of type I collagen.

derline of statistical significance) in subjects carrying allele b compared with wild-type subjects (Table 2).

Discussion

The study was carried out in a group of Polish postmenopausal women from the Lower Silesia region as part of a larger study. Nutritional, metabolic, and genetic aspects of the menopause related to obesity in these women have been published recently [14].

A number of polymorphic variants in the VDR gene have been identified and extensively studied in various populations. Most reports on the genotype–phenotype relationship in the VDR gene have focused on the *BsmI* site. Data gathered so far show that allele b of the VDR gene is more advantageous in terms of bone metabolism, calcium homeostasis, bone accrual during childhood, and bone preservation later in life; conversely, wild-type BB subjects could be more likely to develop osteoporosis [15].

In this preliminary report, the *BsmI* allelic frequency (allele b frequency 0.54, bb genotype of 26.7%) was higher than or similar to those observed in other Caucasian populations [9,11–13], especially Slavic, and in earlier Polish studies [16,17]. For example, in a meta-analysis, the *BsmI* bb genotype frequency was 2% among Asians, 5% among African-Americans, and 17% among Caucasians [15].

The absence of the *BsmI* site has been associated with a small-to-modest decrease in bone mass and a twofold increase in the risk of hip fracture compared with the presence of this site [15]. Thus, we tested all clinical characteristics in our group against a dichotomous value: presence or lack of *BsmI* polymorphism in the VDR gene. Despite the fact that we failed to detect statistically significant differences between the VDR genotypes in BMD, a trend towards better bone parameters in women carrying allele b compared with those carrying the BB wild type in our study is similar to that in previous reports. It could also be confirmed by the higher allele b frequency in the control group (normal bone mass) compared with osteopenic/osteoporotic women. Conceivably, lower activity of the resorption marker ICTP in allele b carriers (at the borderline of statistical significance) could be one factor involved,

Table 2. Differences in clinical and biochemical data between subjects without allele b (wild type) and allele b carriers

	Wild type (BB)	*BsmI* polymorphism (Bb and bb)	*p*
Age (yr)	53.2 ± 3.7	54.3 ± 5.3	0.6
Height (cm)	165.3 ± 5.8	166.8 ± 9.3	0.7
BMI (kg/m^2)	30.0 ± 7.4	29.1 ± 6.9	0.2
Lumbar spine L2–L4			
BMD (g/cm^2)	1.220 ± 0.185	1.271 ± 0.193	0.4
T-score	0.095 ± 1.522	0.468 ± 1.606	0.5
Z-score	0.224 ± 1.314	0.526 ± 1.457	0.4
Femoral neck			
BMD (g/cm^2)	0.942 ± 0.143	0.998 ± 0.133	0.2
T-score	–0.482 ± 1.061	–0.056 ± 1.059	0.2
Z-score	–0.213 ± 0.943	0.0157 ± 0.988	0.3
Ward's triangle			
BMD (g/cm^2)	0.823 ± 0.183	0.875 ± 0.154	0.3
T-score	–0.755 ± 1.365	–0.374 ± 1.160	0.3
Z-score	–0.307 ± 1.218	–0.001 ± 1.066	0.4
Trochanter major			
BMD (g/cm^2)	0.812 ± 0.145	0.867 ± 0.125	0.2
T-score	–0.080 ± 1.173	0.338 ± 1.041	0.2
Z-score	–0.050 ± 1.082	0.296 ± 0.958	0.3
ICTP (ng/mL)	7.5 ± 4.6	3.3 ± 1.2	0.08
Osteocalcin (ng/mL)	20.9 ± 6.0	20.8 ± 10.8	0.9

BMI = body mass index; BMD = bone mineral density; ICTP = carboxyterminal cross-linked telopeptide of type I collagen.

but this requires further more robust studies.

The incidence of osteoporosis and osteopenia was quite low in our group of postmenopausal women compared with other populations, but this was in agreement with our previous observations [17,18]. It seems to reflect the real BMD distribution in the Polish postmenopausal population of Lower Silesia.

A very recent study summarizing various gene polymorphisms involved in osteoporosis and fracture occurrence has indicated associations of a single nucleotide polymorphism and BMD and emphasizes the importance of using ethnically homogenous populations in such epidemiologic studies [19]. Our group, being the offspring of families of various descent from other parts of central and eastern Poland, could not fulfill this criterion. However, our data seem to be interesting and potentially important, so we decided to continue this study.

The discrepancies with earlier studies may be explained not only by the differences in genetic background of the populations studied but, alternatively, environmental–lifestyle factors or gene–environmental/gene–nutrient interaction (e.g. calcium intake), as previously suggested for this polymorphism, could be involved.

We can confirm an association between VDR gene polymorphism and a decreased BMD in Polish postmenopausal women from Lower Silesia, but further more robust studies are required.

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